CLAIMS

1.   A method for identifying a protein with the use of mass spectrometry, characterized in that

the method is a method in which by referring to sequence information about a nucleotide sequence of a genomic gene encoding a full-length amino
5   acid sequence of a peptide chain constituting the known protein, about a nucleotide sequence of a reading frame in mRNA enabling translation of the full-length amino acid sequence, and about a (deduced) full-length amino acid sequence encoded by the nucleotide sequence in regard to known individual proteins, which information is recorded in a database on known proteins, one of
10   the known proteins recorded in the database which is assessed to correspond to a target protein to be analyzed is selected for the , based on a mass spectrometric result actually measured for the target protein to be analyzed,

wherein

(1) the mass spectrometric result actually measured for the target protein
15   is a result obtained from mass spectrometric analysis comprising at least a set of respective actually measured mass values (Mex) of a plurality of peptide fragments determined by

subjecting a peptide chain isolated in advance that constitutes the target protein to be analyzed to reduction treatment capable of cleaving disulfide (S-S)
20   bond in Cys-Cys bond present therein and to treatment that unfolds folding of the target protein to linearize the peptide chain constituting the target protein,

further carrying out treatment for site-specific proteolysis that selectively cleaves a peptide chain at a particular amino acid or amino acid sequence to evenly and selectively prepare a plurality of peptide fragments derived from the
25   linearized peptide chain collected from the target protein, and

determining the respective actually measured mass values (Mex) of the plurality of peptide fragments, based on a result for masses (M) of the plurality of the peptide fragments produced that is measured by mass spectrometry as molecular weights (M+H/Z; Z=1) of corresponding monovalent "parent cation species" or as molecular weights (M-H/Z; Z=1) of corresponding monovalent "parent anion species";

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(2) in regard to known individual proteins recorded in said database on known proteins, referring to sequence information about a nucleotide sequence of a genomic gene encoding a full-length amino acid sequence of a peptide chain constituting the known protein, about a nucleotide sequence of a reading frame in mRNA enabling translation of the full-length amino acid sequence, and about a (deduced) full-length amino acid sequence encoded by the nucleotide sequence,

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calculating predicted molecular weights (Mref) of a plurality of peptide fragments derived from a peptide chain having said full-length amino acid sequence, presumably produced by subjecting the peptide chain having the full-length amino acid sequence that is translated according to the genomic gene encoding the known protein to the reduction treatment for a sulfanyl (-SH) group on a Cys side chain and to the treatment of site-specific proteolysis to create a set of the predicted molecular weights (Mref) of the plurality of predicted peptide fragments derived from the known protein, and

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employing as a reference standard database, a data set of the predicted molecular weights (Mref) of the plurality of peptide fragments, wherein the data set is composed of total sets of the predicted molecular weights (Mref) of the plurality of known protein-derived predicted peptide fragments calculated for all the known individual proteins recorded in the database on known proteins;

50

(3) performing a first comparison operation whereby the set of the respective actually measured mass values (Mex) of the plurality of peptide fragments determined for the target protein to be analyzed is compared with

55   each of the sets of the predicted molecular weights (Mref) of the plurality of known protein-derived predicted peptide fragments calculated for the known individual proteins recorded in the database on known proteins, and

the number of the actually measured peptide fragments derived from the target protein to be analyzed and the number of the known protein-derived

60   predicted peptide fragments judged as having a substantial match between the respective actually measured mass values (Mex) and the predicted molecular weights (Mref) of the plurality of predicted peptide fragments in each of the sets derived from the known proteins in consideration of a measurement error attributed to the utilized mass spectrometry itself are determined each

65   individually for the known proteins comprised in the reference standard database, and

selecting from among the known proteins determined in the first comparison operation, known proteins in decreasing order of the number of the actually measured peptide fragments derived from the target protein to be

70   analyzed and the number of the known protein-derived predicted peptide fragments judged as having a match to classify a known protein exhibiting the highest number of the match into a group of first candidate known protein(s) as a candidate of identification for the target protein to be analyzed; and ˄

(4) when the group of the first candidate known protein(s) comprises one

75   type of known protein, judging the one type of known protein selected from the database as being a single candidate of identification for the target protein to be analyzed.

2.   The method according to claim 1, characterized in that

in the case where in referring to sequence information about the selected known protein judged in the step (4) as being a single candidate of identification for the target protein to be analyzed,

5   the number of actually measured peptide fragments that are derived from the target protein to be analyzed, which are not judged in the first comparison operation of the step (3) as having a match to the predicted molecular weights (Mref) of the plurality of predicted peptide fragments in the set derived from the known protein judged as being a candidate of identification, is zero,

10   the selected known protein judged in the step (4) as being a single candidate of identification for the target protein to be analyzed is judged as being a highly accurate single candidate of identification.

3.   The method according to claim 1, characterized in that

in the case where in referring to sequence information about the selected known protein judged in the step (4) as being a single candidate of identification for the target protein to be analyzed,

5   when arranging the plurality of the actually measured peptide fragments derived from the target protein to be analyzed that are judged in the first comparison operation of the step (3) as having a match to the predicted molecular weights (Mref) of the plurality of predicted peptide fragments in the set derived from the known protein judged as being a candidate of identification,

10   in positions to be occupied by the corresponding predicted peptide fragments derived from the known protein, a group of the actually measured peptide fragments that are judged as having a match constitutes consecutive amino acid sequences that is contained in the full-length amino acid sequence of the known protein,

15   the selected known protein judged in the step (4) as being a single

candidate of identification for the target protein to be analyzed is judged as

being a highly accurate single candidate of identification.

4.   The method according to claim 3, characterized in that

in the case where there remains a unidentified actually measured

peptide fragment derived from the target protein to be analyzed that is not

judged in the first comparison operation of the step (3) as having a match to the

5   predicted molecular weights (Mref) of the plurality of predicted peptide

fragments in the set derived from the known protein judged as being a

candidate of identification, the method further comprises: in regard to the

unidentified actually measured peptide fragment derived from the target protein

to be analyzed,

10   on the assumption that for a group of predicted peptide fragments which

are linked to the consecutive amino acid sequence portions contained in the

full-length amino acid sequence of the known protein, which are derived from

the known protein judged as being a candidate of identification, and which are

unidentified by the corresponding actually measured peptide fragments, there

15   would exist post-translational modification attributed to modifying group addition

to a side chain of an amino acid residue present in the unidentified predicted

peptide fragments, calculating predicted molecular weights (Mref) of predicted

peptide fragments having the post-translational modification attributed to

modifying group addition to a side chain of an amino acid residue; and

20   performing a second comparison operation whereby the presence or

absence of the unidentified actually measured peptide fragment having the

actually measured mass value (Mex) matching to any of the predicted molecular

weights (Mref) of the predicted peptide fragments having the post-translational

modification attributed to modifying group addition is judged, wherein

25   when at least one unidentified actually measured peptide fragment

derived from the target protein to be analyzed having the actually measured

mass value (Mex) matching to any of the predicted molecular weights (Mref) of

the predicted peptide fragments having the post-translational modification

attributed to modifying group addition is selected,

30   the selected known protein judged in the step (4) as being a single

candidate of identification for the target protein to be analyzed is judged as

being a highly accurate single candidate of identification.

5.   The method according to claim 3, characterized in that

in the case where there remains a unidentified actually measured

peptide fragment derived from the target protein to be analyzed that is not

judged in the first comparison operation of the step (3) as having a match to the

5   predicted molecular weights (Mref) of the plurality of predicted peptide

fragments in the set derived from the known protein judged as being a

candidate of identification, the method further comprises: in regard to the

unidentified actually measured peptide fragment derived from the target protein

to be analyzed,

10   on the assumption that for an N-terminal portion of a group of predicted

peptide fragments which are linked to the consecutive amino acid sequence

portions contained in the full-length amino acid sequence of the known protein,

which are derived from the known protein judged as being a candidate of

identification, and which are unidentified by the corresponding actually

15   measured peptide fragments, post-translational processing of N-terminal

truncation would occur to convert the known protein to a mature protein,

calculating predicted molecular weights (Mref) of a plurality of predicted peptide

fragments derived from the post-translational N-terminal processing,

presumably generated by subjecting an assumed amino acid sequence of the

20   known protein to the introduction treatment of a protecting group and to the site-specific proteolytic treatment; and

        performing a second comparison operation whereby the presence or absence of the unidentified actually measured peptide fragment derived from the target protein to be analyzed having the actually measured mass value

25   (Mex) matching to any of the predicted molecular weights (Mref) of the predicted peptide fragments derived from the post-translational N-terminal processing is judged, wherein

        when at least one unidentified actually measured peptide fragment derived from the target protein to be analyzed having the actually measured

30   mass value (Mex) matching to any of the predicted molecular weights (Mref) of the predicted peptide fragments derived from the post-translational N-terminal processing is selected,

        the selected known protein judged in the step (4) as being a single candidate of identification for the target protein to be analyzed is judged as

35   being a highly accurate single candidate of identification.

      6.   The method according to claim 3, characterized in that

        in the case where there remains a unidentified actually measured peptide fragment derived from the target protein to be analyzed that is not judged in the first comparison operation of the step (3) as having a match to the predicted

5   molecular weights (Mref) of the plurality of predicted peptide fragments in the set derived from the known protein judged as being a candidate of identification, the method further comprises: in regard to the unidentified actually measured peptide fragment derived from the target protein to be analyzed,

        on the assumption that for a C-terminal portion of a group of predicted

10   peptide fragments which are linked to the consecutive amino acid sequence portions contained in the full-length amino acid sequence of the known protein,

which are derived from the known protein judged as being a candidate of identification, and which are unidentified by the corresponding actually measured peptide fragments, post-translational processing of C-terminal

15 truncation would occur to convert the known protein to a C-terminally truncated protein, calculating predicted molecular weights (Mref) of a plurality of predicted peptide fragments derived from the post-translational processing of C-terminal truncation, presumably generated by subjecting an assumed amino acid sequence of the known protein to the introduction treatment of a protecting

20 group and to the site-specific proteolytic treatment; and

performing a second comparison operation whereby the presence or absence of the unidentified actually measured peptide fragment derived from the target protein to be analyzed having the actually measured mass value (Mex) matching to any of the predicted molecular weights (Mref) of the

25 predicted peptide fragments derived from the post-translational processing of C-terminal truncation is judged, wherein

when at least one unidentified actually measured peptide fragment derived from the target protein to be analyzed having the actually measured mass value (Mex) matching to any of the predicted molecular weights (Mref) of

30 the predicted peptide fragments derived from the post-translational C-terminal processing is selected,

the selected known protein judged in the step (4) as being a single candidate of identification for the target protein to be analyzed is judged as being a highly accurate single candidate of identification.

7. The method according to claim 3, characterized in that

in the case where there remains a unidentified actually measured peptide fragment derived from the target protein to be analyzed that is not judged in the first comparison operation of the step (3) as having a match to the predicted

5   molecular weights (Mref) of the plurality of predicted peptide fragments in the

set derived from the known protein judged as being a candidate of identification,

the method further comprises: in regard to the unidentified actually measured

peptide fragment derived from the target protein to be analyzed,

on the assumption that in genomic gene portions encoding portions of a

10   group of predicted peptide fragments which are linked to the consecutive amino

acid sequence portions contained in the full-length amino acid sequence of the

known protein, which are derived from the known protein judged as being a

candidate of identification, and which are unidentified by the corresponding

actually measured peptide fragments, splicing different from presumable RNA

15   splicing in a plurality of exons contained in the genomic gene portions would

occur, calculating predicted molecular weights (Mref) of a plurality of predicted

peptide fragments derived from the alternative splicing, presumably generated

by subjecting an assumed amino acid sequence of the known protein to the

introduction treatment of a protecting group and to the site-specific proteolytic

20   treatment; and

performing a second comparison operation whereby the presence or

absence of the unidentified actually measured peptide fragment derived from

the target protein to be analyzed having the actually measured mass value

(Mex) matching to any of the predicted molecular weights (Mref) of the

25   predicted peptide fragments derived from the alternative splicing is judged,

wherein

when at least one unidentified actually measured peptide fragment

derived from the target protein to be analyzed having the actually measured

mass value (Mex) matching to any of the predicted molecular weights (Mref) of

30   the predicted peptide fragments derived from the alternative splicing is selected,

the selected known protein judged in the step (4) as being a single candidate of identification for the target protein to be analyzed is judged as being a highly accurate single candidate of identification.

8.   The method according to claim 3, characterized in that

in the case where there remains a unidentified actually measured peptide fragment derived from the target protein to be analyzed that is not judged in the first comparison operation of the step (3) as having a match to the predicted

5   molecular weights (Mref) of the plurality of predicted peptide fragments in the set derived from the known protein judged as being a candidate of identification, the method further comprises: in regard to the unidentified actually measured peptide fragment derived from the target protein to be analyzed,

on the assumption that in portions of a group of predicted peptide

10   fragments which are linked to the consecutive amino acid sequence portions contained in the full-length amino acid sequence of the known protein, which are derived from the known protein judged as being a candidate of identification, and which are unidentified by the corresponding actually measured peptide fragments, protein splicing that removes a portion of an amino acid sequence

15   thereof would occur, calculating predicted molecular weights (Mref) of a plurality of predicted peptide fragments derived from the protein splicing, presumably generated by subjecting an assumed amino acid sequence of the known protein to the introduction treatment of a protecting group and to the site-specific proteolytic treatment; and

20   performing a second comparison operation whereby the presence or absence of the unidentified actually measured peptide fragment derived from the target protein to be analyzed having the actually measured mass value (Mex) matching to any of the predicted molecular weights (Mref) of the predicted peptide fragments derived from the protein splicing is judged, wherein

25   when at least one unidentified actually measured peptide fragment

derived from the target protein to be analyzed having the actually measured

mass value (Mex) matching to any of the predicted molecular weights (Mref) of

the predicted peptide fragments derived from the protein splicing is selected,

the selected known protein judged in the step (4) as being a single

30   candidate of identification for the target protein to be analyzed is judged as

being a highly accurate single candidate of identification.

9.   The method according to claim 3, characterized in that

in the case where there remains a unidentified actually measured peptide

fragment derived from the target protein to be analyzed that is not judged in the

first comparison operation of the step (3) as having a match to the predicted

5   molecular weights (Mref) of the plurality of predicted peptide fragments in the

set derived from the known protein judged as being a candidate of identification,

the method further comprises: in regard to the unidentified actually measured

peptide fragment derived from the target protein to be analyzed,

on the assumption that for genomic gene portions encoding a group of

10   predicted peptide fragments which are linked to the consecutive amino acid

sequence portions contained in the full-length amino acid sequence of the

known protein, which are derived from the known protein judged as being a

candidate of identification, and which are unidentified by the corresponding

actually measured peptide fragments, one replacement of a translated amino

15   acid attributed to single nucleotide polymorphism would occur in an exon

contained in the genomic gene portions, calculating predicted molecular weights

(Mref) of a plurality of predicted peptide fragments derived from the amino acid

replacement of single nucleotide polymorphism, presumably generated by

subjecting an assumed amino acid sequence of the known protein to the

20   introduction treatment of a protecting group and to the site-specific proteolytic treatment; and

performing a second comparison operation whereby the presence or absence of the unidentified actually measured peptide fragment derived from the target protein to be analyzed having the actually measured mass value

25   (Mex) matching to any of the predicted molecular weights (Mref) of the predicted peptide fragments derived from the amino acid replacement of single nucleotide polymorphism is judged, wherein

when at least one unidentified actually measured peptide fragment derived from the target protein to be analyzed having the actually measured

30   mass value (Mex) matching to any of the predicted molecular weights (Mref) of the predicted peptide fragments derived from the amino acid replacement of single nucleotide polymorphism is selected,

the selected known protein judged in the step (4) as being a single candidate of identification for the target protein to be analyzed is judged as

35   being a highly accurate single candidate of identification.

10.   The method according to claim 1, characterized in that

in the case where in referring to sequence information about the selected known protein judged in the step (4) as being a single candidate of identification for the target protein to be analyzed, and

5   arranging the plurality of the actually measured peptide fragments derived from the target protein to be analyzed that are judged in the first comparison operation of the step (3) as having a match to the predicted molecular weights (Mref) of the plurality of predicted peptide fragments in the set derived from the known protein judged as being a candidate of identification,

10   in positions to be occupied by the corresponding predicted peptide fragments derived from the known protein,

a group of the actually measured peptide fragments that is judged as

having a match constitutes consecutive amino acid sequences contained in the

full-length amino acid sequence of the known protein except for positions to be

15   occupied by some predicted peptide fragments,

the selected known protein judged in the step (4) as being a single

candidate of identification for the target protein to be analyzed is judged as

being a highly accurate single candidate of identification.

11.   The method according to claim 10, characterized in that

in the case where there remains a unidentified actually measured peptide

fragment derived from the target protein to be analyzed that is not judged in the

first comparison operation of the step (3) as having a match to the predicted

5   molecular weights (Mref) of the plurality of predicted peptide fragments in the

set derived from the known protein judged as being a candidate of identification,

the method further comprises: in regard to the unidentified actually measured

peptide fragment derived from the target protein to be analyzed,

on the assumption that for a group of predicted peptide fragments which

10   are located within the consecutive amino acid sequences portions contained in

the full-length amino acid sequence of the known protein, which are derived

from the known protein judged as being a candidate of identification, and which

are unidentified by the corresponding actually measured peptide fragments,

there would exist post-translational modification attributed to modifying group

15   addition to a side chain of an amino acid residue present in the unidentified

predicted peptide fragments, calculating predicted molecular weights (Mref) of

predicted peptide fragments having the post-translational modification attributed

to modifying group addition to a side chain of an amino acid residue; and

performing a second comparison operation whereby the presence or

20   absence of the unidentified actually measured peptide fragment derived from

the target protein to be analyzed having the actually measured mass value

(Mex) matching to any of the predicted molecular weights (Mref) of the

predicted peptide fragments having the post-translational modification attributed

to modifying group addition is judged, wherein

25   when at least one unidentified actually measured peptide fragment

derived from the target protein to be analyzed having the actually measured

mass value (Mex) matching to any of the predicted molecular weights (Mref) of

the predicted peptide fragments having the post-translational modification

attributed to modifying group addition is selected,

30   the selected known protein judged in the step (4) as being a single

candidate of identification for the target protein to be analyzed is judged as

being a highly accurate single candidate of identification.

12.   The method according to claim 10, characterized in that

        in the case where there remains a unidentified actually measured peptide

fragment derived from the target protein to be analyzed that is not judged in the

first comparison operation of the step (3) as having a match to the predicted

5   molecular weights (Mref) of the plurality of predicted peptide fragments in the

set derived from the known protein judged as being a candidate of identification,

the method further comprises: in regard to the unidentified actually measured

peptide fragment derived from the target protein to be analyzed,

        on the assumption that in genomic gene portions encoding portions of a

10   group of predicted peptide fragments in an internal unidentified region which are

located within the consecutive amino acid sequence portions contained in the

full-length amino acid sequence of the known protein, which are derived from

the known protein judged as being a candidate of identification, and which are

unidentified by the corresponding actually measured peptide fragments, splicing

15   different from presumable RNA splicing in a plurality of exons contained in the

genomic gene portions would occur, calculating predicted molecular weights

(Mref) of a plurality of predicted peptide fragments derived from the alternative

splicing, presumably generated by subjecting an assumed amino acid sequence

of the known protein to the introduction treatment of a protecting group and to

20   the site-specific proteolytic treatment; and

performing a second comparison operation whereby the presence or

absence of the unidentified actually measured peptide fragment derived from

the target protein to be analyzed having the actually measured mass value

(Mex) matching to any of the predicted molecular weights (Mref) of the

25   predicted peptide fragments derived from the different splicing is judged,

wherein

when at least one unidentified actually measured peptide fragment

derived from the target protein to be analyzed having the actually measured

mass value (Mex) matching to any of the predicted molecular weights (Mref) of

30   the predicted peptide fragments derived from the alternative splicing is selected,

the selected known protein judged in the step (4) as being a single

candidate of identification for the target protein to be analyzed is judged as

being a highly accurate single candidate of identification.

13.   The method according to claim 10, characterized in that

in the case where there remains a unidentified actually measured peptide

fragment derived from the target protein to be analyzed that is not judged in the

first comparison operation of the step (3) as having a match to the predicted

5   molecular weights (Mref) of the plurality of predicted peptide fragments in the

set derived from the known protein judged as being a candidate of identification,

the method further comprises: in regard to the unidentified actually measured

peptide fragment derived from the target protein to be analyzed,

on the assumption that in portions of a group of predicted peptide

10 fragments in an internal unidentified region which are located within the

consecutive amino acid sequence portions contained in the full-length amino

acid sequence of the known protein, which are derived from the known protein

judged as being a candidate of identification, and which are unidentified by the

corresponding actually measured peptide fragments, protein splicing that

15 removes a portion of an amino acid sequence thereof would occur, calculating

predicted molecular weights (Mref) of a plurality of predicted peptide fragments

derived from the protein splicing, presumably generated by subjecting an

assumed amino acid sequence of the known protein to the introduction

treatment of a protecting group and to the site-specific proteolytic treatment;

20 and

performing a second comparison operation whereby the presence or

absence of the unidentified actually measured peptide fragment derived from

the target protein to be analyzed having the actually measured mass value

(Mex) matching to any of the predicted molecular weights (Mref) of the

25 predicted peptide fragments derived from the protein splicing is judged, wherein

when at least one unidentified actually measured peptide fragment

derived from the target protein to be analyzed having the actually measured

mass value (Mex) matching to any of the predicted molecular weights (Mref) of

the predicted peptide fragments derived from the protein splicing is selected,

30 - the selected known protein judged in the step (4) as being a single

candidate of identification for the target protein to be analyzed is judged as

being a highly accurate single candidate of identification.

14. The method according to claim 10, characterized in that

in the case where there remains a unidentified actually measured peptide

fragment derived from the target protein to be analyzed that is not judged in the

first comparison operation of the step (3) as having a match to the predicted

5   molecular weights (Mref) of the plurality of predicted peptide fragments in the

set derived from the known protein judged as being a candidate of identification,

the method further comprises: in regard to the unidentified actually measured

peptide fragment derived from the target protein to be analyzed,

on the assumption that for genomic gene portions encoding respective

10   portions of a group of predicted peptide fragments in an internal unidentified

region which are located within the consecutive amino acid sequence portions

contained in the full-length amino acid sequence of the known protein, which

are derived from the known protein judged as being a candidate of identification,

and which are unidentified by the corresponding actually measured peptide

15   fragments, one substitution of a translated amino acid attributed to single

nucleotide polymorphism would occur in an exon contained in the genomic

gene portions, calculating predicted molecular weights (Mref) of a plurality of

predicted peptide fragments derived from the amino acid substitution of single

nucleotide polymorphism, presumably generated by subjecting an assumed

20   amino acid sequence of the known protein to the introduction treatment of a

protecting group and to the site-specific proteolytic treatment; and

performing a second comparison operation whereby the presence or

absence of the unidentified actually measured peptide fragment derived from

the target protein to be analyzed having the actually measured mass value

25   (Mex) matching to any of the predicted molecular weights (Mref) of the

predicted peptide fragments derived from the amino acid substitution of single

nucleotide polymorphism is judged, wherein

when at least one unidentified actually measured peptide fragment

derived from the target protein to be analyzed having the actually measured

30   mass value (Mex) matching to any of the predicted molecular weights (Mref) of

the predicted peptide fragments derived from the amino acid substitution of single nucleotide polymorphism is selected,

the selected known protein judged in the step (4) as being a single candidate of identification for the target protein to be analyzed is judged as

35 being a highly accurate single candidate of identification.

15. The method according to any one of claims 4 to 9 and 11 to 14, characterized in that

the method further comprises: at least in the second comparison operation,

5 utilizing as the mass spectrometric result actually measured for the target protein to be analyzed,

in addition to the set of the respective actually measured mass values (Mex) of the plurality of peptide fragments that are determined based on a result for masses (M) of the plurality of generated peptide fragments measured by

10 mass spectrometry as molecular weights (M+H/Z; Z=1) of corresponding monovalent "parent cation species" or as molecular weights (M-H/Z; Z=1) of corresponding monovalent "parent anion species",

also at least a result of molecular weights of fragmented derivative ion species measured by MS/MS analysis for the actually measured peptide

15 fragment derived from the target protein to be analyzed that is judged in the first comparison operation as being the unidentified actually measured peptide fragment derived from the target protein to be analyzed as "daughter ion species" derived from the "parent cation species" of the peptide fragment or as "daughter ion species" derived from the "parent anion species" of the peptide

20 fragment;

in regard to the actually measured peptide fragment derived from the target protein to be analyzed newly selected in the second comparison

operation as being the unidentified actually measured peptide fragment derived

from the target protein to be analyzed having the actually measured mass value

25   (Mex) matching to any of the predicted molecular weights (Mref) of the

predicted peptide fragments,

performing comparison whereby molecular weights of fragmented

derivative ion species presumably generated in MS/MS analysis due to the

assumed amino acid sequence and additional modification group constituting

30   the corresponding predicted peptide fragment are also compared with the

actually measured result of the molecular weights of the fragmented derivative

ion species for the actually measured peptide fragment derived from the target

protein to be analyzed; and

when correspondence relationship is also confirmed at least between the

35   actually measured result of the molecular weights of the fragmented derivative

ion species for the actually measured peptide fragment derived from the target

protein to be analyzed and the predicted values of the molecular weights of the

predicted fragmented derivative ion species for the corresponding predicted

peptide fragment,

40   regarding as judgment with high accuracy, the judgment of the actually

measured peptide fragment derived from the target protein to be analyzed

selected in the second comparison operation, wherein .

the selected known protein judged in the step (4) as being a single

candidate of identification for the target protein to be analyzed is judged as

45   being a highly accurate single candidate of identification.

16.   The method according to any one of claims 1 to 15, characterized in that

the method further comprises prior to the site-specific proteolytic

treatment, performing on the linearized peptide chain, selective introduction of a

protecting group for the sulfanyl (-SH) group on the Cys side chain, to prepare

5   the resulting linearized peptide chain having the protected Cys.